

abbreviation. Applicant further states that "Bn" is the abbreviation for "benzyl" and state that this is found on page 40, lines 10-24 and scheme (Fig. 2) and Scheme 3 (Fig. 3). In this case the abbreviation is found in Figure 2 without the definition and is not found in Figure 3. Applicant should provide either a reference in the specification where these abbreviations are defined or else a reference in the prior art using these abbreviations where the definitions are given.

Office Action at 2.

Applicants wish to correct their previous assertion that "MOM" stands for "methoxymethoxy." MOM stands for "methoxymethyl." Applicants reiterate that "Bn" is an abbreviation for "benzyl." Furthermore, as requested, Applicants have provided as Appendix II a prior art reference using these abbreviations and defining them. The reference is to Protective Groups in Organic Synthesis, Second Edition, Theodora W. Greene and Peter G.M. Wuts (Eds.), John Wiley & Sons, Inc., New York, 1992 which states these abbreviations and their definitions at page 17 ("2. Methoxymethyl Ether (MOM Ether)") and at page 47 ("42. Benzyl Ether (BnOR)").

The Examiner also raised a further objection regarding Table 2:

In Table 2, it was asked what "4", " ± 0.05 ", " ± 180 ", " ± 0.07 ", " ± 540 ", ... " ± 710 ", " ± 407 " mean in the second, forth, sixth and eighth lines. Although applicants did not answer this question, upon further review it is concluded that these are the standard errors of the values above them. However, it is not understood why " \pm " is after the values above in some cases and with the standard errors in others. Applicants have satisfactorily explained the first part of the previous question regarding the fifth and ninth columns.

Office Action at 2-3.

Applicants respectfully point out that they did answer the question raised above by pointing to footnote "a" in Table 2. This footnote refers back to footnote "a" in Table 1 which stated that the values in the second rows of some columns represent the "mean standard errors from sets of six replicates." See response mailed July 23, 2001 at 5. Applicants point out that the placement of the \pm as after the values above in some cases and with the standard errors in others reflects the formatting of the table. In columns having adequate space to accommodate the standard error on the same line as the reported value (i.e., columns 4 and 5), the value and standard error are reported on the same line. In the remaining columns, the standard error is reported below the value.

The Examiner also raised an objection with respect to the notations used to indicate the chemical names of the compounds:

In Table A provided with the amendment as an explanation of the chemical names of the compounds, the structures are listed as "(R)-1a", "(S)-1a", etc. Figures 2 and 4 and Tables 1-3 use the notation "N62C-a", etc. It is presumed that "(R)-1a" is meant to be the (R) isomer of the "a" cysteine change at a given position, e.g. N62. The "1a" is not found except for the 3 structures in the upper right hand corner of Fig. 3 and in several places in the specification such as page 30, lines 3 and 26, page 31, line 28, page 32, line 28, etc. This discrepancy is not understood.

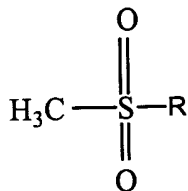
Office Action at 3.

The Examiner's presumption that "(R)-1a" means the (R) isomer of the "a" cysteine change at a given position, e.g., N62 is correct. Applicants respectfully submit that there is no discrepancy and illustrate the consistent use of the objected-to notation by way of an example.

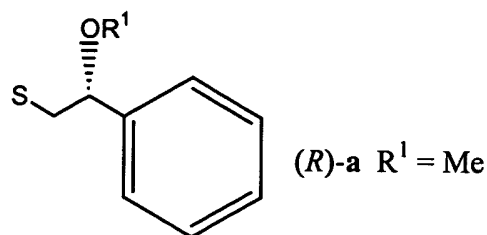
In Fig. 2, the top line represents derivitization of a *Bacillus lentus* subtilisin enzyme ("SBL") that has been mutated to include the amino acid cysteine to provide a free sulfhydryl group ("SH") on the enzyme. The mutated enzyme is represented by the structure at the left of the top line in Fig. 2. as:



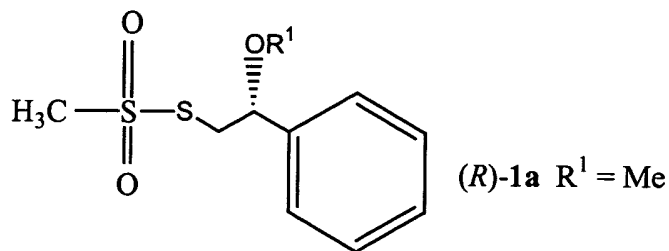
The structure above the arrow in the top line of Fig. 2:



generically represents various methanethiosulfonate reagents **1a-i** used to derivatize the mutated *Bacillus lentus* subtilisin enzyme. The methanethiosulfonate moiety (CH₃SO₂) is common to all these reagents, while different moieties are represented by the "R" in the structure above the arrow. The various R groups contained within structures **1a-i** are illustrated in the remaining part of Fig. 2. For example, the R group associated with structure **1a** is illustrated by the structure on the left side of the second row of Fig. 2:



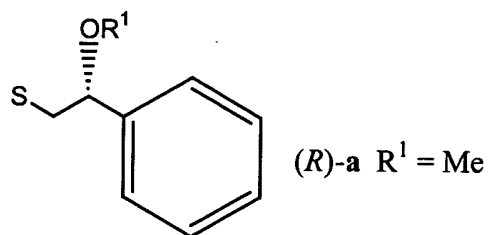
Substituting in the (R)-a group shown in the left side of the second row of Fig. 2 into the rest of the structure shown above the arrow in the top row of Fig. 2 leads to methanethiosulfonate structure (R)-1a:



The free sulfhydryl from the mutated *Bacillus lentus* subtilisin enzyme and the methanethiosulfonate reagent (R)-1a undergo an $\text{S}_\text{N}2$ reaction displacing the SO_2CH_3 group from (R)-1a to generate the chemically modified enzyme diagrammed in Fig. 2 as:



where R is the moiety formerly attached to the methanethiosulfonate reagent, illustrated in Fig. 2 as:



Thus a mutated SBL derivatized with (R)-1a has the following structure:

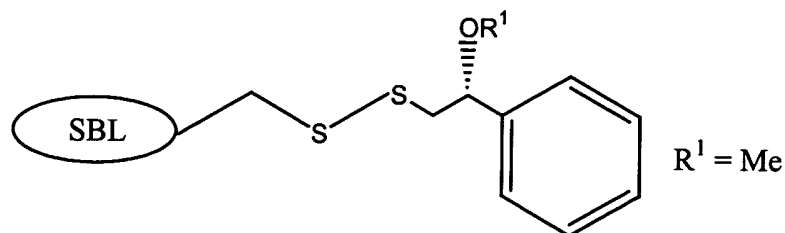
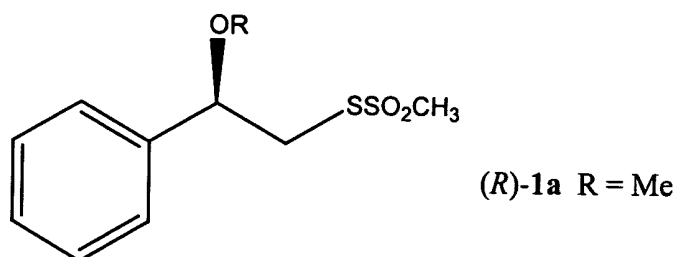


Fig. 3 illustrates, *inter alia*, the synthesis used for generating (R)-1a, the structure of which is shown in the upper right hand of Fig. 3 as:



which, of course, is the same as the first structure 1a (from Fig. 2) illustrated above, but viewed from behind. Thus, consistent notation is used in the specification and drawings and Applicants respectfully request that the objection be withdrawn.

Rejection Under 35 U.S.C. § 112

The following 112, first paragraph scope rejection was made by the Examiner:

Claims 1-11, 13, 51-57, 59 and 61-62 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for Bacillus lentus subtilisin N62C-a, N62C-d, N62C-e, N62C-f (R isomer only), N62C-h, N62C-i (R isomer only), S166C-d (S isomer only), S166C-h (S isomer only), S166-I (S isomer only), L217C-a, L217C-b, L217C-d, L217C-e, L217C-f and L217C-i, does not reasonably provide enablement for the scope of the instant claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims. The instant mutations give an increase in activity for amidase and/or esterase above the standard error of the wild type while the other mutations do not (Table 1). The other mutations would not have a utility as they would have less activity than the wild type. Note, N62C-c, S166C-c and L217C-c and the modifications discussed in Example 2 are omitted from the list supra because they do not meet the requirement of claim 1 that the thiol side chain is chiral.

Office Action at 3.

During the interview, it was determined that the specification does enable a person skilled in the art to use the invention commensurate in scope with the pending claims. The standard for determining whether the specification meets the enablement requirement is whether the experimentation needed to practice the invention is undue or unreasonable. *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988). Support for this

determination is set forth below organized according to the *Wands* factors with emphasis placed on those implicated by the Examiner's rejection.

The breadth of the claims

As was determined during the interview, the breadth of the claims is limited with respect to: (1) the type of enzyme (modified serine hydrolases), (2) the location of the cysteine amino acid replacement (at a subsite), and (3) the nature of the substituent group replacing the cysteine thiol hydrogen (a chiral substituent).

The nature of the invention

As was determined during the interview, the invention relates to modified serine hydrolases (and methods for producing same) wherein said hydrolases comprise a cysteine residue substituted for an amino acid in a subsite, and the cysteine is modified by replacing the thiol hydrogen with a substituent group to provide a thiol side chain comprising a chiral substituent.

The state of the prior art

The prior art provides techniques needed for making the serine hydrolase cysteine mutants, and for modifying the cysteine to replace the thiol hydrogen with a substituent group to provide a thiol side chain comprising a chiral substituent.

Level of skill in the art

As was determined during the interview, there is a high level of skill in the protein engineering (molecular biology and biochemical arts) and in the synthetic organic chemistry arts to which the claimed invention pertains. Where different arts are involved in the invention, the specification is enabling if it enables persons skilled in each art to carry out the aspect of the invention applicable to their specialty. *In re Naquin*, 398 F.2d 863, 866, 158 USPQ 317, 319 (CCPA 1968). A person of ordinary skill in the protein engineering arts would readily be able to use the teachings of the specification to generate and express cysteine mutants at desired places. *See, e.g.*, spec. at 12:26-17:2. Similarly, a synthetic organic chemist would readily be able to use the teachings of the specification to make and couple chiral substituents to the cysteine mutants. *See, e.g.*, specification at, *e.g.*, 18:8-19:2, 26:9-27:12, 33:3-44:26.

Level of predictability in the art

As was determined in the interview, the protein engineering arts involve some degree of unpredictability with respect to the function of the engineered proteins. The scope of the required enablement varies inversely with the degree of predictability involved, but even in unpredictable arts, a disclosure of every operable species is not required. *See* MPEP § 2164.04 (August 2001). The extensive guidance provided by the specification, the high level of skill in the art, and the results illustrated by the working examples sufficiently offset the unpredictability of the art. The claimed invention therefore is fully enabled.

The amount of teaching or guidance provided by the specification

As was determined during the interview, the specification teaches one of ordinary skill how to use the claimed invention for catalyzing the attachment of a chiral moiety to a substrate to generate an enantiomerically biased product. *See, e.g.*, specification at 5:8-18, spec at 17:7-18:7; 21:21-22:8, and Example 1 (25:4 *et seq.*) The specification further teaches how to use enzymes that have less than wild-type esterase and amidase activity and improved amidase/esterase ratios to catalyze peptide bond formation from an ester substrate. *See, e.g.*, specification at 21:14-20. Examples of such enzymes from those listed in Table 1 (specification at 27:25 *et seq.*) having lower than wild-type amidase or esterase activity, and better than wild-type amidase/esterase ratios are listed in the table below.

Enzyme	Esterase k_{cat}/K_M (mM ⁻¹ s ⁻¹)	Amidase k_{cat}/K_M (mM ⁻¹ s ⁻¹)	Esterase/Amidase Ratio
wt	3560	209	17
S166-a(S)	1246	26	48
S166-b(S)	929	15	62
S166-d(R)	4215	75	56
S166-e(R)	4076	101	40
S166-e(S)	3964	64	62
S166-f(R)	1495	22	68
S166-f(S)	3277	52	63
S166-g(R)	4281	104	41
S166-g(S)	4069	37	109
S166-h(R)	2150	35	61
S166-i(R)	1488	20	74

The specification also provides guidance on how to make and use embodiments other than those specifically exemplified by the working examples. The specification provides guidance on selecting preferred sites for targeting the chemical modifications (*i.e.*, S₁, S₁', or S₂) (*see* specification at 3:13-29) (sites defined by the specification at 7:14-19), selecting preferred serine hydrolases for practicing the invention (*see, e.g.*, specification at 11:1-12); selecting residues for modification using structural homology information between SBL and other enzymes (*see, e.g.*, specification at 11:13-12:25). The latter cite also includes identification

of specific residues for modification in subtilisin-type serine hydrolases, trypsin-chymotrypsin type serine hydrolases, and alpha/beta serine hydrolases.

Thus, the specification provides adequate guidance on how to make and use the claimed invention for a variety of applications, including guidance on how to use embodiments of the claimed invention having less than wild-type esterase and amidase activity, and improved esterase/amidase ratios.

The number of working examples

As was determined during the interview, the specification contains working examples illustrating the making and testing of 50 different embodiments of the claimed invention. *See* specification at pages 27-28. The working examples teach that 25 of these diastereomeric enzymes have improved amidase or esterase activities (as identified by Examiner Patterson on page 3 of the Office Action), and another 11 have improved esterase/amidase ratios as compared to the wild type enzyme (*See* Table above). The working examples therefore illustrate the making and using of 36 different embodiments falling within the scope of the claims. The number of working examples is comparable to what the CCPA previously has found enabling for generic claims. *See, e.g., In re Angstadt*, 537 F.2d, 498, 502-503, 190 USPQ 214, 218 (CCPA 1976)(stating that applicants "are not required to disclose every species encompassed by their claims even in an unpredictable art" and finding that disclosure of forty working examples [*cf.* instant disclosure of 36 operative examples] sufficiently described subject matter of claims).

Quantity of experimentation

As was determined during the interview, the quantity of experimentation needed to practice the full scope of the invention is not undue, but instead is routine in the protein engineering arts. "The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)(citing *In re Angstadt*, 537 F.2d 489, 502-504, 190 USPQ 214, 217-219 (CCPA 1976)). The experimentation involves making compositions according to the guidance provided by the specification (as described above), and testing them. Testing may include routine amidase, esterase, and stereoselectivity assays such as those taught by the specification. *See, e.g.,* specification at 19:3-21:12, 26:5-7, and at 27:13-28:10.

As determined in the interview, and summarized in view of the foregoing, Applicants respectfully submit that the full scope of the claims is enabled by the specification and request withdrawal of the rejection under 35 U.S.C. section 112, first paragraph, and reconsideration of the claims.

Rejection Under 35 U.S.C. § 103(a)

Each of the claims at issue also stands rejected under 35 U.S.C. section 103(a):

Claims 1-11, 13, 51-57, 59 and 61-62 are rejected under 35 U.S.C. 103(a) as being unpatentable over Berglund, et al. (A or C2), Davis, et al. (C3) or DeSantis, et al. (C4). . . . It would have been obvious to one of ordinary skill in the art to modify serine proteases as done in the instant references and assay them for activity, absent unexpected results. The motivation would have been to obtain different mutant enzymes that may have some use. . . . Whether or not the chemical side chain alteration are chiral or not do not affect this rejection. Wide variations are obtained with both chiral and non-chiral changes.

Office Action at 4.

References C3 and C4 (Davis, et al. (1998), & DeSantis, et al.(1998)) are Applicants' disclosure of their own work within the year before the application filing date and so cannot be used against Applicants. *In re Katz* 687 F.2d 450, 214 USPQ 14 (CCPA 1982). Applicants file with this response a *Katz* declaration establishing that these articles describe Applicants' own work.² See MPEP § 2132.01 (August 2001).

The remaining references fail to teach or suggest "chiral substituent" limitation

As was established during the interview, the Examiner has not made out a *prima facie* case of obviousness. "To establish *prima facie* obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art." *In re Royka*, 490 F.2d 981, 985, 180 USPQ 580, 583 (CCPA 1974). Emphasis added. MPEP § 2143.03 (August 2001). Each of the two independent claims at issue (amended claims 1 (composition) and 51 (method)) includes a claim limitation restricting the scope of the claim to a modified serine hydrolase (claim 1) or a method for producing a modified serine hydrolase (claim 55) that incorporates "a thiol side chain comprising a *chiral substituent*."

Berglund et al. (A) teach three thiol non-chiral side chains. *See id.* at 2508. Berglund et al. (C2) teach eleven non-chiral thiol side chains. *See id.* at 5266. Because neither Berglund et al. reference (A or C2), teaches or suggests the "thiol side chain comprising a chiral substituent" limitation, no *prima facie* obviousness case has been made out. Thus, as was determined during the interview, withdrawal of the rejection under 35 U.S.C. section 103(a) is proper and reconsideration of the claims is respectfully requested.

CONCLUSION

Applicants respectfully request reconsideration of the claims in view of the

² Richard C. Lloyd, a co-author of the Davis, et al. (1998) reference was inadvertently omitted as an inventor on the instant case. That error has been corrected by a filing accompanying this response.

remarks made herein. A notice of allowance is earnestly solicited. In the event that a telephonic interview would be helpful for advancing the prosecution, the Examiner is invited to contact the undersigned at (415) 393-2651.

DATE: April 3, 2001

Respectfully submitted,

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APPENDIX II

PROTECTIVE GROUPS IN ORGANIC SYNTHESIS.

Second Edition

THEODORA W. GREENE

The Rowland Institute for Science, Inc.

and

PETER G. M. WUTS

The Upjohn Company

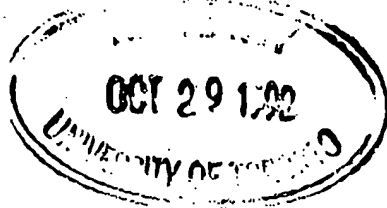


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PREFACE



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Library of Congress Cataloging in Publication Data:

Greene, Theodora W., 1931-

Protective groups in organic synthesis / Theodora W. Greene and Peter G. M. Wuts. -- 2nd ed.

p. cm.

Includes index.

ISBN 0-471-62301-6

1. Organic compounds--Synthesis. 2. Protective groups (Chemistry)

I. Wuts, Peter G. M.

QD262.G665 1991

547.2--dc20

90-45708
CIP

Printed in the United States of America

10 9 8 7 6 5 4 3

Printed and bound by Courier Companies, Inc.

Since publication of the groups and many new groups have been developed. Differences have been added. To conserve space age reactions have been explanatory comments, information has been obtained. Limitations. For example, protective groups only if they complete through 1989. are included for 1990.

Two new sections on protection for the amide amines because their chemistry of protection identical to those in the of each protective group

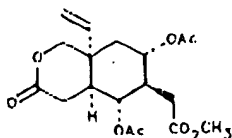
A number of people completing this project. I am which provided many recommendations, Spencer Knapp, amide protection. I thank for spelling and consistencies to make sure my

INCLUDING 1,2- AND 1,3-DIOLS

This is the standard method for unhindered and unhindered alcohols.

A number of methods have been reported for the cleavage of Me_3Si since Me_3Si reagent also cleaves many other ethers. It can be maintained by control of the reaction conditions.

These are not cleaved under these conditions.



Most commonly used method for the cleavage of ethers with a variety of structures.

82% yield.^{20,21}

In this case the 6-O-methyl group is cleaved to give methylated glucose.

82% yield.²¹

95-98% yield.²⁴

84% yield.²⁵ In this case the acetate group that can be hydrolyzed.

The methyl ether is converted to an alcohol with complete racemization.

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Collect. Vol. V, 245 (1973).

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Substituted Methyl Ethers

2. Methoxymethyl Ether (MOM Ether): $\text{CH}_3\text{OCH}_2\text{-OR}$ (Chart 1)

Formation

1. $\text{CH}_3\text{OCH}_2\text{Cl}$, NaH, THF, 80% yield.¹
2. $\text{CH}_3\text{OCH}_2\text{Cl}$, $i\text{-Pr}_2\text{NEt}$, 0° , 1 h $\rightarrow 25^\circ$, 8 h, 86% yield.² This is the most commonly employed procedure for introduction of the MOM group. The reagent chloromethyl methyl ether is reported to be carcinogenic.
3. $\text{CH}_2(\text{OMe})_2$, cat. P_2O_5 , CHCl_3 , 25° , 30 min, 95% yield.³
4. $\text{CH}_2(\text{OMe})_2$, Me_3SiH or $\text{CH}_2=\text{CHCH}_2\text{SiMe}_3$, I_2 , 76-95% yield.⁴

INCLUDING 1,2- AND 1,3-DIOLS

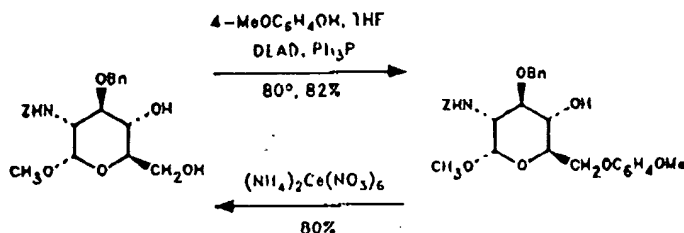
I. Navia, *Carbohydr. Res.*, **174**, 265*Tetrahedron Lett.*, **30**, 4669 (1989).

1966)

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Chem. Commun., 277 (1974); R. Gigg, *J.*Kloosterman, and J. H. Van Boom, *J.*Koning, and J. H. van Boom, *Synthesis*.C. D. Warren and R. W. Jeanloz, *Car-*
h. K. Tachi, and K. Fukuzumi, *J. Org.*
kel and J. H. van Boom, *Tetrahedron Lett.*,m., **4**, 441 (1985)*Int. Ed. Engl.*, **15**, 558 (1976).*Lett.*, 2885 (1970).*Chem., Sect. B*, **25B**, 658 (1986).i. Visapic, *Tetrahedron Lett.*, 3753 (1975).M. F. Sinibaldi, and Y. Troin, *Tetrahedron*

OR

 $p\text{-ClC}_6\text{H}_4\text{OR}$ his synthesis to minimize ring sulfonation
concentrated $\text{H}_2\text{SO}_4/\text{AcOH}$.¹n *Chem. Soc.*, **90**, 1090 (1968). $\text{C}_6\text{H}_5\text{OR}$ THF, 82-99% yield.^{1,2}

Z = benzyloxycarbonyl, DEAD = diethyl azodicarboxylate

Cleavage

1. Ceric ammonium nitrate, CH_3CN , H_2O (4:1), 0° , 10 min, 80-85% yield.^{1,2} This group is stable to 3N HCl, 100° ; 3N NaOH, 100° ; H_2 , 1200 psi; O_3 , MeOH, -78° ; Raney Ni, 100° ; LiAlH_4 ; Jones reagent and pyridinium chlorochromate (PCC).

1. T. Fukuyama, A. A. Laud, and J. M. Hotchkiss, *Tetrahedron Lett.*, **26**, 6291 (1985).
2. M. Petitou, P. Duchaussoy, and J. Choay, *Tetrahedron Lett.*, **29**, 1389 (1988).

41. 2,4-Dinitrophenyl Ether: RODNP : $2,4\text{-(NO}_2)_2\text{-C}_6\text{H}_3\text{OR}$

Formation

1. 2,4-Dinitrofluorobenzene, DABCO, DMF, 85% yield.¹ When this group was used to protect an anomeric center of a carbohydrate, only the β -isomer was formed, but this could be equilibrated to the α -isomer in 90% yield with K_2CO_3 in DMF.

1. H. J. Koeners, A. J. De Kok, C. Romers, and J. H. Van Boom, *Recl. Trav. Chim. Pays-Bas*, **99**, 355 (1980).

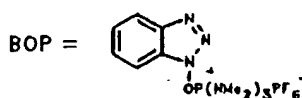
42. Benzyl Ether (BnOR): PhCH_2OR (Chart 1)

Formation

1. BnCl , powdered KOH, $130-140^\circ$, 86% yield.¹
2. BnCl , $\text{Bu}_4\text{N}^+\text{HSO}_4^-$, 50% KOH, benzene.²
3. NaH , THF, BnBr , $\text{Bu}_4\text{N}^+\text{I}^-$, 20° , 3 h, 100%.³ This method was used to protect a hindered hydroxyl group. Increased reactivity is achieved by the *in situ* generation of benzyl iodide.

250 PROTECTION FOR THE CARBOXYL GROUP

73-97% yield²). Phenyl esters are readily cleaved under basic conditions (H_2O_2 , H_2O , DMF, pH 10.5, 20° , 15 min).³



1. I. J. Galpin, P. M. Hardy, G. W. Kenner, J. R. McDermott, R. Ramage, J. H. Seely, and R. G. Tyson, *Tetrahedron*, **35**, 2577 (1979).
2. B. Castro, G. Evin, C. Selve, and R. Seyer, *Synthesis*, 413 (1977).
3. G. W. Kenner and J. H. Seely, *J. Am. Chem. Soc.*, **94**, 3259 (1972).

36. *p*-(Methylmercapto)phenyl Ester: $\text{RCO}_2\text{C}_6\text{H}_4\text{-}p\text{-SCH}_3$

The *p*-(methylmercapto)phenyl ester has been prepared from an *N*-protected amino acid and 4- $\text{CH}_3\text{SC}_6\text{H}_4\text{OH}$ (DCC, CH_2Cl_2 , 0° , 1 h $\rightarrow 20^\circ$, 12 h, 60-70% yield). The *p*-(methylmercapto)phenyl ester serves as an unactivated ester that is activated on oxidation to the sulfone (H_2O_2 , AcOH, 20° , 12 h, 60-80% yield) which then serves as an activated ester in peptide synthesis.¹

1. B. J. Johnson and T. A. Ruettinger, *J. Org. Chem.*, **35**, 255 (1970).

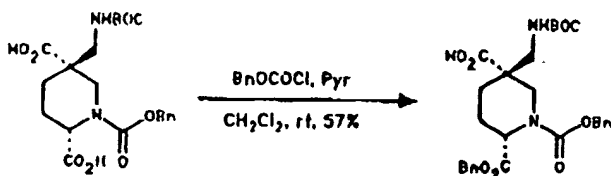
37. Benzyl Ester: $\text{RCO}_2\text{CH}_2\text{C}_6\text{H}_5$, RCO_2Bn (Chart 6)

Formation

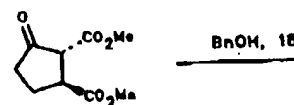
Benzyl esters are readily prepared by many of the classical methods (see introduction to this chapter), as well as by many newer methods, since benzyl alcohol is unhindered and relatively stable to acid.

1. BnOCOCl , Et_3N , 0° , DMAP, CH_2Cl_2 , 30 min, 97% yield.¹

In the case of very hindered acids the yields are poor and formation of the symmetrical anhydride is observed. Useful selectivity can be achieved for a less hindered acid in the presence of a more hindered one.²



2. A methyl ester can be exchanged for h , ---MeOH .³



3. For amino acids: DCC, DMAP, Bn

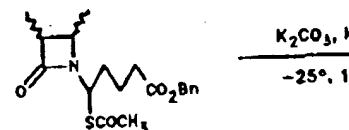
Cleavage

The most useful property of benzyl esters is hydrogenolysis.

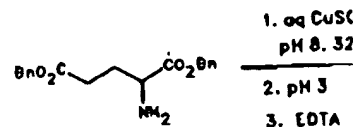
1. $\text{H}_2/\text{Pd-C}$, 25° , 45 min-24 h, high

Catalytic transfer hydrogenation (entries benzyl esters in some compounds that can be cleaved).

2. Pd-C , cyclohexene⁶ or 1,4-cyclohexadiene
3. Pd-C , 4.4% HCOOH , MeOH , 25°
4. K_2CO_3 , H_2O , THF, $0^\circ \rightarrow 25^\circ$, 1



5. AlCl_3 , anisole, CH_2Cl_2 , CH_3NO_2 conditions were used to cleave all derivatives.
6. Na, ammonia, 50% yield.¹¹ The benzyl ester of an amino acid; the Cb also cleaved.
7. Aqueous CuSO_4 , EtOH, pH 8, aminetetraacetic acid), 75% yield



8. Benzyl esters can be cleaved by